

The University of Chicago

THE IRON CONTENT AND OXYGEN CAPACITY OF BLOOD

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY
AND PHARMACOLOGY

1933

By
MARTHA JOHNSON

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By MARTHA JOHNSON

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In the course of studies on the acid-base metabolism of different organs in dogs, it was necessary to know the hemoglobin concentration of arterial and venous blood with an accuracy of 0.1 mm per liter. With the gasometric method of Van Slyke and Neill (1) it was difficult and at times impossible to obtain checking results on the O₂ capacity of venous dog blood, although with arterial blood duplicate analyses agreed usually to within 0.05 mm. As an expedient under these circumstances, the venous O₂ capacities were calculated from the arterial values, the difference in the water content of the two bloods being used (assuming that hemoglobin varies directly with total solids in arterial and venous blood drawn simultaneously from a given animal), and the directly observed venous capacities were always lower than these calculated values, sometimes by as much as 10 per cent. This difficulty was later traced to the fact that after the blood was drawn, it was kept *at its original tension* over mercury for several hours, until the analyses for O₂ and CO₂ content could be completed, before the blood was aerated for the capacity determinations. Neill and Hastings (2) have shown that methemoglobin formation takes place spontaneously when blood is allowed to stand, and that this occurs more rapidly in *partially reduced blood* than in either fully oxygenated or fully reduced blood. We found that, if venous blood is aerated immediately after drawing from an animal, the O₂ capacity values show excellent agreement among duplicates, and also agree with the values for arterial blood having the same water content and drawn simultaneously from the same animal. Also the capacity values on such immediately aerated bloods do

not change when the blood is allowed to stand for 48 hours at refrigerator temperature. Our difficulties with the O₂ capacity determinations on venous blood were therefore due to a failure to realize the importance of *prompt* aeration of the blood after drawing from an animal.

These difficulties with the gasometric oxygen capacity determination stimulated us to seek another method by which the accuracy of the gasometric method could be checked. This paper presents the details of the determination of iron in blood of the required accuracy and shows that the two methods agree to within 1 per cent, the limit of error of the iron method.

A review of the literature of the past 20 years shows many studies (3, 4) on the iron content of blood, but in none of these is the accuracy as great as 1 per cent. Probably the most accurate of these is the work of Blackwood and Stirling (4), in which iron is determined iodometrically in 2 cc. samples of blood. They worked out a set of conditions of acidity and concentration of KI which gave iron values within 2 per cent of the theoretical amount present in standard FeCl₃ solution, when 0.005 Na₂S₂O₃ was used in the titration.

As part of our effort to develop a method for iron in blood which would be accurate to at least 1 per cent, the method was tried of oxidizing the blood with H₂SO₄ and HNO₃, reducing the iron with zinc, and titrating the resulting ferrous sulfate with KMnO₄. We found that it was quite possible to determine iron in standard ferric sulfate solutions with the required accuracy by this method, but that in titrating the iron solutions obtained from oxidized blood, something was present which interfered with the definiteness of the KMnO₄ end-point, so that an accuracy greater than 2 or 3 per cent was impossible. Even though the iron was precipitated as Fe(OH)₃ and redissolved in H₂SO₄ before the reduction and titration, this difficulty could not be avoided.

The method which we finally found to give the required accuracy was an iodometric method which differs from that reported by Blackwood and Stirling (4) in several details, involving particularly control of the concentration of salt as well as of acid during the thiosulfate titration, and a special procedure for the standardization of the thiosulfate solution.

An Iodometric Method for the Determination of Iron in Blood

In general the method is as follows: The blood is oxidized with concentrated H_2SO_4 , concentrated HNO_3 , and 30 per cent H_2O_2 ; the iron is precipitated as ferric hydroxide by the addition of NH_4OH ; the precipitate is collected on a filter, washed free from sulfate, and redissolved in HCl ; the acid solution is partially neutralized with NaOH , KI is added, and the liberated iodine is titrated with $\text{Na}_2\text{S}_2\text{O}_3$.

Reagents—

1. 1.2 N HCl (approximate). Dilute 100 cc. of concentrated HCl to 1 liter with water.

2. 0.6 N HCl (approximate). Dilute 1 volume of the 1.2 N HCl with 1 volume of water.

3. 6 N NaOH (approximate). Dissolve 240 gm. of NaOH in 1 liter of solution.

4. 1 per cent soluble starch. Dissolve 1 gm. of soluble starch in 100 cc. of boiling water and heat for 1 or 2 minutes until the starch is dissolved. Merck's soluble starch (according to Lintner) does not give a red or purple color with I_2 (characteristic of dextrin) and is therefore suitable. This solution should not be used after more than 12 hours standing.

5. 20 per cent KI . Dissolve 20 gm. of KI in 100 cc. of solution. This solution decomposes appreciably in the course of 10 or 12 hours.

6. 0.1 N $\text{Na}_2\text{S}_2\text{O}_3$ (approximate). Dissolve 24.82 gm. of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ in water and make up to 1 liter.

7. 0.0125 N $\text{Na}_2\text{S}_2\text{O}_3$. Dilute 25 cc. of the 0.1 N stock solution of $\text{Na}_2\text{S}_2\text{O}_3$ to 200 cc. This solution is put in the reservoir of a 5 cc. Koch microburette (5) graduated to 0.01 cc., the tip of which is of such a size that 1 drop equals 0.01 cc.

8. Standard 0.01 N FeCl_3 solution. Dissolve 559.2 mg. of an analyzed grade of iron wire (99.89 per cent pure) in 5 cc. of hot concentrated HCl and 20 cc. of water in a standardized 1 liter volumetric flask. When the wire is completely dissolved, gradually add 6 N NaOH with vigorous shaking, during which time the green gelatinous precipitate of $\text{Fe}(\text{OH})_2$ which forms locally is oxidized by the air to ferric ion. The ferric ion remains in solution as FeCl_3 until enough NaOH has been added to neutralize

the acid present, at which time $\text{Fe}(\text{OH})_3$ precipitates. Then add 1 N NaOH a few drops at a time. $\text{Fe}(\text{OH})_2$ is precipitated locally and is oxidized to $\text{Fe}(\text{OH})_3$ by shaking the flask vigorously until the dark brown color disappears, leaving only the red $\text{Fe}(\text{OH})_3$. Continue to add N NaOH in this manner until there is no further darkening of the solution at the point of addition of the alkali due to the formation of $\text{Fe}(\text{OH})_2$. Do not add more than 10 drops of NaOH at a time, since it is difficult to oxidize and to redissolve the precipitate if large amounts of $\text{Fe}(\text{OH})_2$ are formed at one time. Let the suspension of $\text{Fe}(\text{OH})_3$ stand about 1 hour exposed to air. Then add enough concentrated HCl to dissolve completely the $\text{Fe}(\text{OH})_3$ and any small traces of $\text{Fe}(\text{OH})_2$ which may remain unoxidized. Add just enough 6 N NaOH to cause a permanent although not complete precipitation of $\text{Fe}(\text{OH})_3$, and shake the solution with air a final time to insure complete oxidation of ferrous ion. Then add 50 cc. of concentrated HCl, making the final solution 0.6 N with respect to HCl and make up to volume with water.

Procedure

Oxidation of Blood—A 3 or 5 cc. sample of blood, measured with an accuracy of 1 part in 200, is introduced into a 300 cc. Kjeldahl flask, followed by 1 cc. of concentrated H_2SO_4 .

The blood is oxidized by adding four to six successive 0.5 cc. portions of concentrated HNO_3 and heating on a rack over a microburner after each addition. In the early stages of the oxidation, care must be taken not to heat so long between additions of HNO_3 that the charring residue dries where the flame strikes the glass, in order to avoid cracking of the flask. When the charred semisolid residue begins to spatter, another addition of HNO_3 should be made. Later, when the residue no longer contains solid, one heats until white SO_3 fumes appear before the next addition of HNO_3 . When the acid solution appears clear and the only color is that due to the pale yellow solid ferric sulfate, a final addition of HNO_3 is made in such a way that it rinses down any particles which may be adhering to the neck of the flask, and the mixture is heated for about 1 hour until the brown fumes have entirely disappeared, and only white SO_3 fumes remain. Four 0.25 cc. portions of 30 per cent H_2O_2 are added drop by drop

and the mixture heated to the appearance of white fumes after each addition.

The anhydrous solution is cooled, 15 to 20 cc. of distilled water are added, and the solution evaporated over a Bunsen burner until white fumes appear. This evaporation is done to insure the removal of the last traces of HNO_3 . After cooling, about 30 cc. of water are added and the solution is boiled for about 15 minutes to insure complete resolution of the ferric sulfate. When the ferric sulfate is dissolved, the flask is cooled and 1 drop (0.10 to 0.15 cc.) of 0.01 N KMnO_4 is added to the solution. If the pink color remains, the oxidation is complete; if not, the solution is evaporated to white fumes and another 0.5 cc. portion of HNO_3 added and the solution heated to the disappearance of brown fumes, when water is again added, the solution evaporated to white fumes to insure the removal of HNO_3 , and then another 30 cc. portion of water is added, and the mixture heated to dissolve the ferric sulfate. The cooled solution is again tested with KMnO_4 .

Preparation of FeCl_3 Solution for Titration—When the solution is completely oxidized, as evidenced by the permanent KMnO_4 color, 10 cc. of NH_4OH (specific gravity 0.90) are added to the solution in the Kjeldahl flask, which cause the formation of colloidal $\text{Fe}(\text{OH})_3$, as shown by a brown cloudiness. The flask is suspended over a hot-plate for 15 or 20 minutes, during which time the cloudiness disappears, the solution becomes clear and colorless, and a red-brown gelatinous precipitate of $\text{Fe}(\text{OH})_3$ settles out. The precipitate is then collected by filtering the hot ammoniacal solution through a 7 cm. hardened filter paper on a 4 to 5 cm. funnel and is washed about six times with 10 cc. portions of hot ammonia water (1 cc. of concentrated ammonia in 1 liter of water) until a drop of the filtrate no longer shows a positive test for sulfate with 5 per cent BaCl_2 . The funnel is then transferred to another 125 cc. Erlenmeyer flask.

The Kjeldahl flask is rinsed with 10 cc. of the 1.2 N HCl (accurately measured in a volumetric pipette) and this acid is then poured onto the filter paper containing the $\text{Fe}(\text{OH})_3$ precipitate. A second 10 cc. portion of 1.2 N HCl is added in a similar way, first to the Kjeldahl flask and then to the filter paper. This is followed by 10 cc. of 0.6 N HCl . The filter paper is allowed to drain completely between each two additions of acid so as to in-

sure complete removal of the FeCl₃ solution from the filter paper. A second and a third 10 cc. portion of 0.6 N HCl are poured directly on the filter paper from the pipette. The filtrate containing an amount of HCl equivalent to 39 cc. of N HCl is then partially neutralized by the addition of 6 cc. of the 6 N NaOH, leaving approximately 3 cc. of N acid in the 56 cc. of FeCl₃ solution. Although the normality of the HCl and NaOH are only approximately controlled, the volumes used are exactly measured so that all the individuals in a given series of iron determinations have exactly the same acidity and salt content.

Titration with Na₂S₂O₃—5 cc. of the 20 per cent KI are added to the FeCl₃ solution and the mixture is allowed to stand exactly 2 minutes. Then the 0.012 N Na₂S₂O₃ is added from the Koch burette until the brown iodine color has nearly disappeared. 2 drops of the 1 per cent starch solution are added and the mixture turns blue. More of the Na₂S₂O₃ is now added until the solution is colorless. The solution is allowed to stand 5 minutes, during which time the blue color usually returns. A final addition of 1 to 10 drops of Na₂S₂O₃ brings the solution to a permanent endpoint.

Blank determinations are run in exactly the same way on reagents alone. The value of the blank, about 0.05 cc. of 0.01 N thiosulfate, is due almost exclusively to the reagents used in the oxidation of the blood, since a partial blank, run on the reagents used in the precipitation, filtration, and titration, is practically zero.

Standardization of Na₂S₂O₃—1, 3, and 5 cc. samples of the 0.01 N standard FeCl₃ solution are measured with Van Slyke-Ostwald pipettes in 50 cc. Erlenmeyer flasks, the Fe(OH)₃ is precipitated with NH₄OH, the precipitate collected on a filter, washed with hot ammonia water, and then dissolved in exactly 20 cc. of the 1.2 N HCl and exactly 30 cc. of the 0.6 N HCl as described in the procedure given above. This solution is partially neutralized with exactly 6 cc. of 6 N NaOH and the titration with Na₂S₂O₃ carried out as previously described.

The amount of Na₂S₂O₃ equivalent to 1 cc. of FeCl₃ (0.5584 mg. of Fe) varies with the amount of FeCl₃ present, as shown in Table I. Here it is seen that the volume of Na₂S₂O₃ per cc. of FeCl₃ decreases as the amount of FeCl₃ used in the standardization in-

creases from 1 to 10 cc., and the *factors* on the $\text{Na}_2\text{S}_2\text{O}_3$ correspondingly increase. Since these factors are different and since there is not a constant difference between the factors determined with the 1, 3, and 5 cc. samples of FeCl_3 , it is necessary to determine factors on the $\text{Na}_2\text{S}_2\text{O}_3$ by using different amounts of the standard iron solution which have iron contents approximately equal to those of the blood samples. The factor on the $\text{Na}_2\text{S}_2\text{O}_3$ is, then,

TABLE I

Analyses of Standard 0.01 M FeCl_3 , Showing Variations in the Factor on $\text{Na}_2\text{S}_2\text{O}_3$ with Different Amounts of Iron

Date	0.01 M FeCl_3	$\text{Na}_2\text{S}_2\text{O}_3$	$\frac{\text{Cc. Na}_2\text{S}_2\text{O}_3}{\text{Cc. FeCl}_3}$	Factor on $\text{Na}_2\text{S}_2\text{O}_3$
<i>July</i>	<i>cc.</i>	<i>cc.</i>		
9	1	0.805	0.805	1.242
	3	2.408	0.802	1.247
	5	4.010	0.802	1.247
9	1	0.795	0.795	1.258
	3	2.380	0.793	1.261
	5	3.968	0.793	1.261
7	1	0.825	0.825	1.211
	3	2.400	0.800	1.250
	5	3.996	0.799	1.251
	10	7.900	0.790	1.266
1-2	1	0.840	0.840	1.190
	3	2.462	0.820	1.219
	5	4.052	0.810	1.234
1-2	1	0.828	0.828	1.207
	3	2.387	0.799	1.251
	5	3.943	0.788	1.269

defined in terms of the amount of FeCl_3 which is used in the standardization.

If the iron contents of the various blood samples which are being analyzed all fall within a narrow range of a few per cent of each other, as would occur if one were using the same sized samples of blood of nearly the same composition, then one amount of the standard FeCl_3 solution would be sufficient. In general, however, it is safer to use at least two different amounts of the standard iron solution because of the difficulty of knowing beforehand just how much iron is present in the blood. In our work, where 3 to

5 cc. of blood containing about 10 mm per liter of iron were taken, 3 and 5 cc. amounts of the standard 0.01 N FeCl₃ solution served well to define the factors on the Na₂S₂O₃. When the amount of iron in the blood fell between the values of these standards, we interpolated to determine the factor on the Na₂S₂O₃ to be used in calculating the iron content of any particular sample of blood.

Calculation and Data

Four steps are involved in the calculation: calculation of the factors on the thiosulfate for the standard iron solutions and then, by interpolation, for the blood sample; correction for blank on reagents; calculation of the amount of iron (in cc. of 0.01 N, *i.e.* 10⁻⁵ equivalent) in the sample used; and from the size of the sample, calculation of the concentration of iron in the blood, in mm per liter. These are herewith illustrated.

Data—3 cc. of standard 0.01 M FeCl₃ = 2.462 cc. of Na₂S₂O₃. ∴ the Na₂S₂O₃ factor = 1.219, *i.e.* the Na₂S₂O₃ is 0.01219 N.

5 cc. of standard 0.01 M FeCl₃ = 4.052 cc. of Na₂S₂O₃. ∴ the Na₂S₂O₃ factor = 1.234.

3 cc. of blood = 3.076 cc. of Na₂S₂O₃.

Blank on the reagents = 0.045 cc. of Na₂S₂O₃.

By interpolation, the factor on the thiosulfate for the 3 cc. of blood is

$$1.219 + \frac{(3.076 - 2.462)}{(4.052 - 2.462)} (1.234 - 1.219) = 1.225$$

Correcting the Na₂S₂O₃ titer on the blood for the blank, we get 3.076 - 0.045 = 3.031 cc. of Na₂S₂O₃, net for 3 cc. of blood.

Then 3.031 × 1.225 = 3.713 cc. of 0.01 M iron in 3 cc. of blood.

Finally $\frac{3.713}{3} = 1.238 \times 10^{-5}$ equivalents of iron in 1 cc. of blood, which is 12.38 mm of iron per liter of blood.

The accuracy of the method is illustrated by the data in Table II, from an experiment in which determinations were made on mixtures of blood and standard FeCl₃ solutions in addition to those on blood alone. It is seen that in those cases where standard FeCl₃ was added to blood, the equivalents of iron are equal to the sum of the amounts present in each constituent with an accuracy of 0.1 mm per liter.

TABLE II

Analyses of Blood, Standard FeCl₃, and Mixtures of the Two, Showing Recovery of Iron Added to Blood

Sample No.	Blood	0.1 M FeCl ₃	Na ₂ S ₂ O ₃ used	Corrected for blank	× factor	10 ⁻⁵ equivalent of iron	Fe per liter of blood
	cc.	cc.	cc.	cc.			mM
1	5		4.212	4.167	1.247	5.196	10.39
2	5		4.240	4.195	1.247	5.231	10.46
3	5		4.200	4.155	1.247	5.181	10.36
4	2		1.715	1.670	1.245	2.079	10.39
5	2		1.710	1.665	1.245	2.073	10.36
6	2		1.720	1.675	1.245	2.085	10.42
7	2	3	4.125	4.080	1.247	5.088	
8	2	3	4.124	4.079	1.247	5.087	
9	2	3	4.120	4.075	1.247	5.082	
10	0	3	2.413	2.413	1.247	3.010	
11	0	3	2.413	2.413	1.247	3.010	
		Blank	0.045				
		"	0.045				
		"	0.045				
				Factor on Na ₂ S ₂ O ₃			
		1	0.805	} 1.242			
		1	0.795				
		1	0.805				
		3	2.405	} 1.247			
		3	2.411				
		3	2.408				
		5	4.015	} 1.247			
		5	4.010				
		5	4.005				

The sum of the iron content of 2 cc. of blood, as shown in Samples 4 to 6, and the iron content of 3 cc. of the standard FeCl₃ solution, as shown in Samples 10 and 11 ($2.079 + 3.010 = 5.089$) agrees very well with the iron content of the mixtures of these two as shown in Samples 7 to 9.

DISCUSSION

Necessity of Complete Oxidation of Blood—Complete oxidation of the blood and destruction of the nitrosylsulfuric acid are necessary and are insured by the addition of H₂O₂ and by the addition of KMnO₄, as an indicator, to the aqueous solution at the end of the oxidation process. In most cases, the pink color is permanent after the first addition of KMnO₄. In cases where it was not permanent and a further addition of HNO₃ was made, the second addition of KMnO₄ never failed to give a pink color which was permanent. If more than two additions of KMnO₄ are made, the final Na₂S₂O₃ titration value will be appreciably increased, and it will be necessary to apply a correction which is determined by the use of appropriate blanks.

If oxidation is incomplete, the colloidal suspension of Fe(OH)₃ is difficult to break and quantitative separation of Fe(OH)₃ on the filter paper is not possible. Occasionally, the alkaline filtrate from the Fe(OH)₃ is clear but slightly yellow, indicating, probably, incomplete oxidation, although in the few cases where such a filtrate was encountered, no test for iron was found when the filtrate was acidified and tested with potassium thiocyanate.

Necessity of Controlling Salt Concentration and Acidity—That sulfates, phosphates, and acidity are factors to be controlled in order to get accuracy in a thiosulfate titration is well known (4, 6), but attention has not heretofore been called to the fact that other salts, notably chlorides, exert effect. Numerous experiments on this question have led to the following conclusions.

If too much acid is present (for example 39 cc. of N HCl in 50 cc. of solution, the minimal amount necessary to dissolve and wash the ferric hydroxide from the filter paper), the starch-iodine color is purple instead of blue, and agreeing values among duplicates cannot be realized. Therefore, part of this excess acid must be neutralized by NaOH.

For a given amount of ferric ion, the amount of thiosulfate necessary to reach an end-point increases with increasing sulfate concentration and also with increasing acidity; but the thiosulfate required decreases as chlorides are increased, NaCl having a greater effect than the same weight of NH₄Cl. These effects of salts and acids on the thiosulfate titer are much greater when ferric ion is being determined than when KIO₃·HIO₃ is the oxidizing agent and iron is absent.

The amount of thiosulfate required per unit amount of iron (at controlled acidity and salt content) varies with the amount of iron, being smaller with larger amounts of iron. Thus the factor on the thiosulfate increases as the amount of iron increases. The magnitude of this effect has varied considerably in different experiments, although its direction has always been the same (see Table I). It may be that further study would allow one to control the factor on the thiosulfate and make it agree with that found with a $\text{KIO}_3 \cdot \text{HIO}_3$ standardization; until then, however, we feel it safer to carry out our standardization with standard FeCl_3 solution, keeping acidity and salt content exactly the same in both standards and unknowns, and to calibrate the factors on the thiosulfate for each blood sample according to the quantity of thiosulfate used for that sample.

Details of Attaining End-Point with $\text{Na}_2\text{S}_2\text{O}_3$ —It was found that when $\text{Na}_2\text{S}_2\text{O}_3$ was added to the FeCl_3 solution as soon as possible after the addition of KI, the end-point, although sharp, did not persist for more than a few seconds, as was evidenced by the reappearance of the blue color. When the solution was titrated to an end-point with starch and was then allowed to stand 5 minutes and again titrated to the end-point, the blue color did not reappear within 45 minutes to 2 hours. The second end-point was taken as the final one in these cases. It was thought that the reaction between the ferric and iodide ions was incomplete after the first addition of $\text{Na}_2\text{S}_2\text{O}_3$ had been made and that the reappearance of color after the first or preliminary end-point was due to the completion of this reaction; so, a study was made of the effect of allowing various time intervals to elapse after the addition of the KI, before the $\text{Na}_2\text{S}_2\text{O}_3$ was added. The results (not detailed here) showed that the reaction between the ferric and iodide ions is a slow one, and noticeably incomplete in 2 minutes, in the presence of small amounts of iodine such as are present in reaching the preliminary end-point with the iodine as an indicator. The reaction becomes complete for practical purposes by extending the time interval to 5 minutes in the presence of these minute amounts of iodine; or the iodine may be completely removed by attaining the preliminary end-point in the presence of starch, in which case completion of the reaction is practically immediate. From these studies, we decided to add starch in attaining the preliminary end-point.

Studies were also made to find out whether a larger concentration of KI would serve to decrease the time of the reaction. With 10 cc. (instead of 5 cc.) of 20 per cent KI the reaction was complete at the preliminary end-point (that is, no additional color appeared on standing), but the magnitude of the titration values increased progressively as the time interval varied from 15 seconds to 10 minutes. When, then, still larger amounts of KI were used (5 and 10 cc. of 40 per cent KI) in order to see whether these titers could be made to reach a constant maximum in a short time, a red, rather than a blue, appeared on the addition of starch, and this color was not readily discharged during the further addition of Na₂S₂O₃.

From these studies it was concluded that, for the best accuracy, 5 cc. of 20 per cent KI should be used in solutions containing the iron equivalent of 5 cc. of blood or less, that the solutions should be allowed to stand exactly 2 minutes before the Na₂S₂O₃ is added, and that after reaching a preliminary end-point in the presence of starch, 5 minutes be allowed before observing the final end-point.

Accuracy of Method—Table II shows that the maximum difference among triplicates is 0.1 mm when the total hemoglobin is about 10 mm. To obtain this accuracy, the maximum error of any determination should not be greater than 1 part in 200 and that this is realizable seems justified from the following considerations. The error in sampling need not be greater than 1 part in 1000, and the error in the Na₂S₂O₃ titration not greater than 0.01 cc. when the total number of cc. is between 3 and 5, or 1 part in 300 to 500. The other most likely sources of error are incomplete oxidation, loss of material during the frequent transfers and during the oxidation of blood, and inequality of salt or acid concentration in the solution during the titration. These can all be kept below 1 part in 200 by careful manipulation. While the maximum difference among triplicates in Table II is 1 part in 100, the average error is less than one-half of this, as has been borne out by other observations with this method.

Correlation of Iron Content and Oxygen Capacity

Table III shows the extent to which mm per liter of O₂ determined by the Van Slyke-Neill method agree with mm per liter of

iron determined by the iodometric method presented above. The values for mm of iron are averages of triplicate determinations, except in such instances as Sample AN, where one of the triplicates varies from the other two by more than 0.1 mm, in which case the iron value is the average of duplicates. The iron values are from -0.01 to $+0.13$ mm different from the O_2 values,

TABLE III
Comparison of O_2 Capacity and Iron Content of Dog Blood

Sample	O_2	Fe	Average Fe	Difference between Fe and O_2
	mm	mm	mm	mm
A, May 26	10.10	10.23 10.25 10.23	10.23	+0.13
V, " 26	9.90	9.92 9.94 9.96	9.94	+0.04
AN, " 30	9.16	9.36 9.16 9.13	9.15	-0.01
CE, " 30	8.39	8.42 8.45	8.44	+0.05
June 19	10.28	10.39 10.36 10.42	10.39	+0.11
" 19	10.28	10.46 10.39 10.36	10.40	+0.12

The O_2 capacity values are corrected for 0.25 mm of dissolved O_2 (7).

showing that within the limit of experimental error the two values agree.

SUMMARY

An iodometric method for the accurate determination of iron in blood is described by which the iron content may be determined to 0.1 mm of iron per liter of blood. It is shown that the following conditions must be observed in order to attain this accuracy: (1) Care must be taken to insure complete oxidation of the blood. (2) For the $Na_2S_2O_3$ titration, sulfates and phosphates must be removed, the acidity must not be too great, and the acidity and

salt concentration must be accurately controlled. This is accomplished by precipitating the iron as $\text{Fe}(\text{OH})_3$, washing until salts are completely removed, redissolving the iron in a measured excess of HCl , and partially neutralizing the acid with a measured amount of NaOH . (3) The factor on the $\text{Na}_2\text{S}_2\text{O}_3$ must be determined by titration against standard FeCl_3 , the same conditions of salt and acid concentrations as in the unknowns being used. No other standard may be used because the factor on the $\text{Na}_2\text{S}_2\text{O}_3$ varies with the amount of iron. (4) The end-point must be determined in exactly the same way in all samples, standard and unknown, with respect to the time intervals allowed and the amounts of KI used. A preliminary end-point is reached in the presence of starch, and after standing 5 minutes, the final end-point is attained.

The iron content and O₂ capacity of blood were found to agree within 1 per cent.

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